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THE DEPENDENCE OF PIGMENT GRANULE MIGRATION ON THE CORTICAL REACTION IN THE EGGS OF ARBACIA PUNCTULATA [1]

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The migration of pigment granules (echinochrome chromatophores) to the surface of *Arbacia* eggs about 10 minutes after fertilization is a conspicuous event which marks successful initiation of development in this species. These pigment granules are scattered throughout the cytoplasm in the unfertilized egg. Even before fertilization, they move about in the cytoplasm as rapidly as 5 microns per second (Parpart, 1953). Upon fertilization, they migrate to the fertilized egg surface (McClendon, 1909, 1910), which is characterized by the presence of a hyaline layer and fertilization membrane and where a new cortical gel layer will form just beneath the surface.

In sea urchin eggs, elevation of the fertilization membrane is preceded by a wave of cortical granule breakdown (E. N. Harvey, 1911; Moser, 1939). This wave, usually referred to as the cortical reaction, can now be interrupted or blocked by any of three separate methods in order to study the interaction of fertilized and unfertilized cortex on the one hand, with endoplasm on the other (Allen, 1954; Allen and Hagström, 1955a; Hagström and Allen, 1956). The present study was undertaken to determine whether pigment granule migration occurs as a specific interaction between these cytoplasmic granules and fertilized, but not unfertilized egg surface (cf. preliminary note by Allen and Rowe, 1955).

Material and Methods

Arbacia punctulata gametes were obtained by electrical stimulation (E. B. Harvey, 1952). Eggs were deprived of jelly by brief treatment with acid sea water (pH about 5). Insemination was carried out at a sperm concentration of 3×10^6 sperm per ml. and at 20–22° C.

Aliquots of freshly inseminated eggs were transferred at intervals of a few seconds to 0.001% sodium lauryl sulfate in sea water at 30–32° C. for a period of about two minutes, and then were allowed to cool before being washed in sea water.

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Similar treatment of unfertilized eggs did not visibly affect their early development when they were subsequently inseminated. Fertilized eggs were similarly unaffected by this treatment when it was applied after the cortical reaction changes were complete. A small percentage of the eggs (usually less than 20%) exposed to elevated temperature and detergent during the cortical reaction showed interruption of cortical granule breakdown, restricted membrane elevation and other characteristics of partially-fertilized eggs (Allen, 1954; Allen and Hagström, 1955a; Hagström and Allen, 1956). These eggs were isolated from the others and observed for the pattern of pigment distribution and stage of development in which they were arrested.

Other eggs were inseminated in quartz capillaries; a combination of stretching and warming to about 26° with unfiltered light from the microscope lamp sufficed to interrupt the cortical reaction. These cylindrical, partially-fertilized eggs were also followed for changes in pattern of pigment distribution and for other indications of early development changes.

Results

Whether the pigment granules have migrated to the surface or not is best observed under intense illumination. Fertilized eggs with pigment migration complete exhibit a dense, peripheral, pigmented shell (ring in optical section); on the other hand, eggs with blocked cortical reaction show a brilliant red *zone* on the fertilized surface (Fig. 1); the rest of the cytoplasm contains scattered pigment granules as in the unfertilized egg. Near the red zones on the partially-fertilized eggs the endoplasm lacks pigment granules. Examination with a 50 × water-immersion lens (Leitz) of that portion of the surface to which the pigment migrated shows local loss of cortical granules and the establishment of a hyaline layer and blister-like fertilization membrane. The rest of the egg surface remains re-fertilizable for at least two hours, and a second insemination during this time results in polyspermy.

The migration of pigment granules from an extensive area of endoplasm bordering on the fertilized cortex suggested the recruitment of granules from a considerable distance. Furthermore, the intense color of the pigment at the fertilized surface of a partially-fertilized egg suggested that this surface had "attracted" a greater share of the total supply of pigment granules than an equal amount of surface in a totally fertilized egg. To test this possibility, several eggs were rendered partially fertilized while in the shape of cylinders with rounded ends in a quartz capillary of small diameter. On Figure 2, it is possible to see the regular sub-surface arrangement of pigment granules in the fertilized part, and the scattered distribution in the unfertilized part of the egg furthest from the point of sperm entry. Note, however, the intensely pigmented ring at the border of the unfertilized and fertilized cortex. It can also be seen in this figure that the width of the zone of unfertilized cytoplasm from which pigment granules were recruited was about 26 microns.

In control experiments, a few eggs were observed over the course of the summer, which responded to detergent alone without insemination. These eggs must have become "partially-activated." The fact that pigment migrated to the activated regions of cortex in these eggs indicated that the mere presence of sperm was not a factor in this migration of pigment.

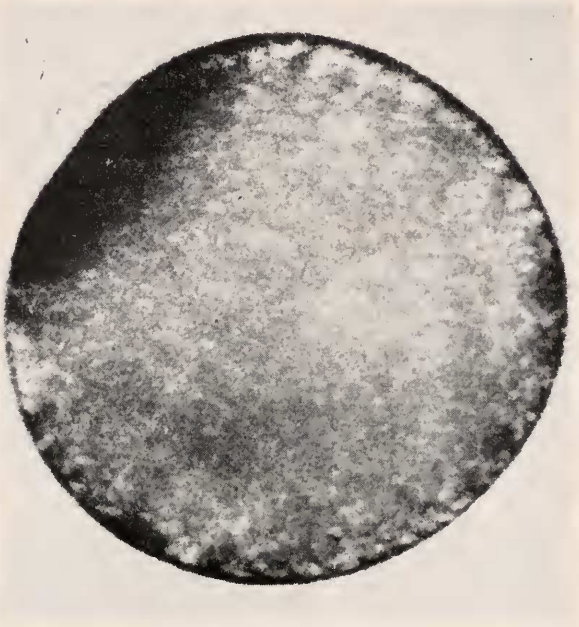

FIGURE 1. A partially-fertilized egg of *Arbacia punctulata* photographed with Kodachrome film and later printed in green light to show the accumulation of pigment (dark) at the region of the egg surface which has undergone a cortical reaction.

Several hundred partially fertilized eggs were observed to determine whether there was any relationship in this species between the amount of cortex affected by the cortical reaction and the extent of development. Such a relationship was clearly evident. Only a few eggs with more than 50% of their surfaces affected by the cortical reaction were observed. (This is probably due in part to the extremely short time that the eggs pass through this condition; *cf.* Figure 1 in Rothschild and

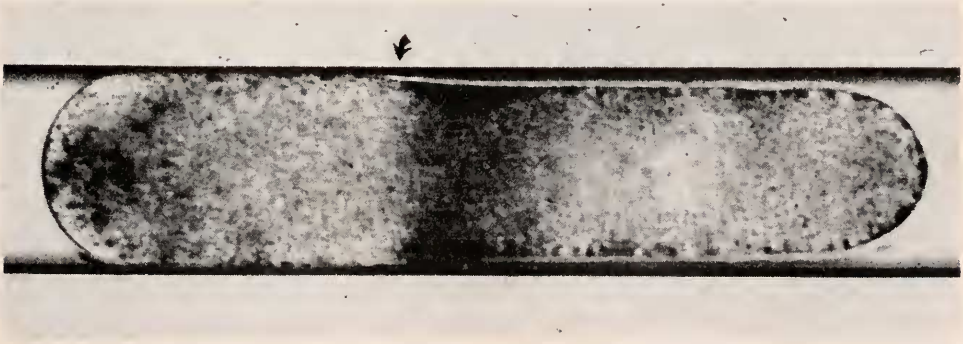

FIGURE 2. Reproduced from Kodachrome as in Figure 1. A partially-fertilized egg obtained by the capillary method (see text). Note the marked accumulation of pigment at the border of fertilized and unfertilized cortex (arrow). This pigment had migrated from nearby unfertilized cytoplasm.

Swann, 1949). Only those eggs with about half of their surface fertilized developed as far as cleavage, and they lacked hyaline layers so that their blastomeres fell apart (*cf.* Herbst, 1900). Eggs with much less than one half their surface fertilized showed varying degrees of development before arrest. The most common stages of arrested development observed after the partial fertilization were metaphase of cleavage, prophase (swollen synkaryon), and varying stages in arrested nuclear migration or fusion. Table I will illustrate by five specific examples the situation often

TABLE I

Examples showing the relation between the portion of the egg surface affected by the cortical reaction and the extent of development in those eggs.
(*For further description, see text.*)

Percentage of surface area covered by the cortical reaction	Stage in which development was arrested
7.7%	Nuclear migration but no fusion
4.5	Deformed spindle
1.2 (sperm penetrated near egg nucleus)	Nuclei completed copulation path but failed to become centered
1.2 (sperm penetrated 170° from surface nearest egg nucleus)	Sperm nucleus completed copulation path, but no migration of egg nucleus
0.4	Sperm nucleus penetrated 5 microns, no further change in location of nuclei, but nuclear swelling about the time of prophase

seen in eggs with arrested development showing extremely small amounts of fertilized cortex but containing a sperm nucleus.

DISCUSSION

It is clear from the foregoing evidence that the migration of pigment granules to the cortex after fertilization is the result of an interaction between fertilized cortex and neighboring endoplasm. In previous studies with related species, it was noted that although fertilized and unfertilized cytoplasm become mixed to a significant extent in spherical partially-fertilized eggs, such mixing is very restricted in cylindrical partially-fertilized eggs obtained by the capillary method (Allen, 1954; Allen and Hagström, 1955a; Allen and Hagström, 1955b). These conclusions were based on light-scattering differences which reveal changes in the endoplasm brought about after fertilization. Unfortunately, the presence of pigment in *Arbacia* renders a parallel study with this species impossible. The recruitment of pigment granules from 26 microns deep within cytoplasm that would otherwise be judged unfertilized (Allen and Hagström, 1955a) suggests that the interaction between fertilized cortex and pigment may be more far-reaching than interaction resulting in light-scattering changes.

The mechanism of pigment granule migration under the influence of fertilized

cortex remains obscure. The observations of Parpart (1953) with the television microscope suggest that invisible fibrils may extend from the cortex to the individual pigment granules. Such a mechanism would almost necessarily have to be invoked to explain the rapid movement which he observed, especially when this movement sometimes moved perpendicular to the direction of cytoplasmic flow.

Summary

1. The intensely pigmented echinochrome granules (chromatophores) of the *Arbacia* egg, which are distributed throughout the endoplasm prior to fertilization, migrate to the fertilized surface about 10 minutes after insemination.

2. In partially-fertilized eggs, in which the cortical reaction has been blocked or interrupted, the pigment granules migrate only to fertilized cortex.

3. Fertilized cortex in partially-fertilized eggs can recruit pigment granules not only from its immediately-underlying endoplasm, but also from a considerable distance in endoplasm apparently otherwise unaffected by the cortical reaction.

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